

AD_____

Award Number: W81XWH-08-1-0180

TITLE: Targeting Fatty Acid Synthase Gene for Prostate Cancer Therapy

PRINCIPAL INVESTIGATOR:

Eiji Furuta, Ph.D.(P.I.)

Kounosuke Watabe, Ph.D. (Mentor)

CONTRACTING ORGANIZATION: Southern Illinois University
Springfield, IL 62794-9626

REPORT DATE: October 2008

TYPE OF REPORT: ANNUAL SUMMARY

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 01/10/2008		2. REPORT TYPE ANNUAL SUMMARY		3. DATES COVERED (From - To) 1 Apr 2008- 30 Sep 2008	
4. TITLE AND SUBTITLE Targeting Fatty Acid Synthase Gene for Prostate Cancer Therapy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-08-1-0180	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Eiji Furuta, Ph.D. (P.I.) Kounosuke Watabe, Ph.D. (Mentor)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Southern Illinois University, Springfield, IL 62794-9626				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012 *				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Fatty acid synthase (FAS) is significantly over-expressed in prostate tumor cells and inhibition of FAS results in apoptosis, suggesting that FAS is an ideal target for drug development. The overarching hypothesis of this project is that a specific inhibitor for FAS dimerization will block the function of this enzyme and cause apoptosis of the tumor cell. Our specific aims are (i) to characterize the apoptotic pathway induced by FAS inhibition, and (ii) to identify small chemical compounds that specifically inhibit dimer formation of FAS enzyme. During the last four months (Apr. 1-July 31, 2008) we mainly focused our effort on the second specific aim and screened a compound library provided by Developmental Therapeutics Program of NCI. We also screened a library of natural products that particularly focused on marine lives and found that one of the natural products in the library showed strong activity of inhibiting FAS. We also found that the purified compound, Cacalol, significantly blocked the activity and expression of FAS. Our results suggest that this compound has a potential utility for the treatment of prostate cancer.					
15. SUBJECT TERMS None Listed.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1
Key Research Accomplishments.....	2
Reportable Outcomes.....	2
Conclusion.....	2
References.....	3
Appendices.....	4

Introduction

Prostate cancer is the second most common malignancy in adult males as well as the second leading cause of cancer-related deaths in the United States. However, virtually no treatment option is available for patients at an advanced stage due to the fact that none of the existing chemotherapeutic agents are effective to treat this type of cancer. Therefore, we essentially need a new approach to define specific target molecules with well-defined clinical rationale so that we will have a better chance of developing a more effective therapeutic agent. Fatty acid synthase (FAS) is significantly over-expressed in prostate tumor cells and inhibition of FAS results in apoptosis, suggesting that FAS is an ideal target for drug development. Although the detailed mechanism of apoptosis induced by FAS inhibition is yet to be elucidated, our preliminary data indicate that (i) FAS is over-expressed in human prostate cancer and this high-expression correlated with poor survival of patients, (ii) specific inhibition of FAS expression by siRNA significantly increased ceramide, and (iii) the high level of ceramide resulted in induction of pro-apoptotic genes, BNIP3, DAPK2 and TRAIL followed by apoptosis. In order to identify an effective inhibitor of FAS, we have also developed a novel high-throughput screening system to block dimerization of FAS enzyme in the hope that screened drugs can be used for the treatment of prostate cancer patients. We plan to test the hypothesis that a specific inhibitor for FAS dimerization will block the function of this enzyme and cause apoptosis of the tumor cell. Our specific aims are (i) to characterize the apoptotic pathway induced by FAS inhibition, and (ii) to identify small chemical compounds that specifically inhibit dimer formation of FAS enzyme. Our effort for the last 4 months (Apr. 1 –July 31) has been mainly focused on the second specific aim.

Body

Our working hypothesis for Specific aim 1 is that apoptosis induced by FAS inhibition is caused by the suppression of CPT1 followed by accumulation of ceramide which in turn activates pro-apoptotic genes, BNIP3, TRAIL and DAPK2. We found that inhibition of FAS by chemical inhibitor or shRNA resulted in accumulation of ceramide followed by apoptosis and that three pro-apoptotic genes, BNIP3, TRAIL and DAP kinase 2, were significantly up-regulated in response to the FAS inhibition (1-3). These lines of evidence strongly support our hypothesis that apoptosis induced by the FAS inhibition is caused by suppression of CPT1 followed by accumulation of ceramide which in turn activates pro-apoptotic genes, BNIP3, TRAIL and DAPK2. We are also currently performing immunohistochemical analysis to examine the level of CPT1, FAS, BNIP3, TRAIL and DAP kinase 2 in clinical specimens from prostate cancer patients. The results of these experiments will provide us with critical information to understand the mechanism of apoptosis induced by FAS inhibition.

In Specific aim 2, we hypothesize that a specific inhibitor for FAS dimerization will block the function of this enzyme and cause apoptosis of the tumor cell. For this purpose, we are currently screening the compound library provided by Developmental Therapeutics Program of NCI/NIH. This program has more than 200,000 compounds that are available to us for screening. We are also screening a library of natural products (obtained from Marine Biology Institute in Japan (Kamaishi city)) that is particularly focused on marine lives. We found that one of the natural products in the library showed strong activity of inhibiting FAS. This product inhibited both the activity and expression of FAS. The activity was purified by TLC followed by HPLC. We also found that this compound is identical with Cacalol. Our results of in vivo experiment using a xenograft mouse model indicate that Cacalol has strong inhibitory activity to tumor growth. These results suggest that this compound has a potential utility for the treatment of prostate cancer. We are currently examining the exact mechanism of FAS inhibition by Cacalol by analyzing inhibitory kinetics of Cacalol using purified FAS enzyme.

Key Research Accomplishments

1. We found that inhibition of FAS can cause apoptosis through the up-regulation of pro-apoptotic genes, BNIP3, TRAIL and DAP kinase
2. Our preliminary data indicate that the expression of FAS and BNIP3 is inversely correlated in prostate cancer.
3. We found that Cacalol significantly inhibits both activity and expression of FAS.

Reportable Outcomes

At this point, we do not have any reportable outcomes mainly due to the limited time period (Apr.1-July31).

Conclusion

Our results strongly support the hypothesis that apoptosis induced by FAS inhibition is caused by suppression of CPT1 followed by accumulation of ceramide which in turn activates pro-apoptotic genes, BNIP3, TRAIL and DAPK2. Further analysis of this pathway may reveal a more detailed mechanism of apoptosis induced by FAS inhibition which may lead to the identification of a better target to block the FAS pathway. We indeed found a compound called Cacalol which significantly inhibits the FAS activity. This compound may have potential utility for the treatment of prostate cancer.

“So what”

The most significant finding during this period is the discovery of Cacalol as an inhibitor of FAS enzyme and this compound indeed blocks tumor growth in vivo. Although it needs further analysis for the detailed mechanism of inhibition, Cacalol may serve as a lead compound to identify a potent anti-cancer drug which can be tested for a clinical trial in the future.

References

1. Bandyopadhyay, S., Sudha K Pai, Misako Watabe, Steven C Gross, Shigeru Hirota, Sadahiro Hosobe, Taisei Tsukada, Kunio Miura, Ken Saito, Stephen J Markwell, Ying Wang, and Kounosuke Watabe (2005) FAS expression inversely correlates with PTEN level in prostate cancer and a PI 3-kinase inhibitor synergizes with FAS siRNA to induce apoptosis. *Oncogene*, **24**, 5389-5395
2. Bandyopadhyay, S., Zhan, R., Wang, Y., Pai, SK., Hirota, S., Hosobe, S., Takano, Y., Saito, K., Furuta, E., Iizumi, M., Mohinta, S., Watabe, M., Chalfant C., and Watabe, K. (2006) Mechanism of apoptosis induced by the inhibition of Fatty Acid Synthase in breast cancer cells. *Cancer Res*, **66**, 5934-5940.
3. Furuta, E., Pai, SK., Zhan, R., Bandyopadhyay, S., Watabe, M., Mo, Y-Y., Hirota, S., Hosobe, S., Tsukada, T., Miura, K., Kamada, S., Saito, K., Iizumi, M., Liu, W., Ericsson, J. and Watabe, K. (2008) Fatty acid synthase gene is up-regulated by hypoxia via activation of Akt and sterol regulatory element binding protein-1. *Cancer Res*. **68**, 1003-1011.

Appendices

N/A